

AMENDED CLAIMS

AP20 Rec'd PCT/PTO 13 FEB 2006

[received by the International Bureau on 11 January 2005 (11.01.05);
Claims 19, 21, 22 and 36 amended; claims 1-18, 20, 23-35, 37-45 unchanged;
(7 pages)]

1. A process for producing an aldehyde derivative of a sialic acid in which a starting material having a sialic acid unit at the reducing terminal is subjected to sequential steps of:

- 5 a) reduction to reductively open the ring at the reducing terminal sialic acid unit, whereby a vicinal diol group is formed; and
 b) selective oxidation to oxidise the vicinal diol group to form an aldehyde group.

2. A process according to claim 1 in which the sialic acid unit at
10 the reducing terminal is joined to the adjacent unit through the 8 carbon atom whereby in step b) the 6,7 vicinal diol group is oxidised to form an aldehyde on the carbon-7 atom.

3. A process according to claim 1 or claim 2 in which the starting material has a terminal saccharide unit at the non-reducing end which has a
15 vicinal diol group and in which the starting material is subjected to a preliminary step, prior to step a), of selective oxidation to oxidise the vicinal diol group to an aldehyde, whereby in step a) the aldehyde is also reduced to form a hydroxy group which is not part of a vicinal diol group.

4. A process according to claim 3 in which the saccharide unit at
20 the non-reducing end is a sialic acid unit.

5. A process according to any preceding claim in which the starting material is a di-, oligo- or poly-saccharide.

6. A process according to claim 5 in which the polysaccharide is a polysialic acid consisting substantially only of units of sialic acid.

25 7. A process according to claim 6 in which the polysaccharide has at least 2, preferably at least 5 or more preferably at least 10, most preferably at least 50 sialic acid units in the molecule.

8. A process according to claim 2 and any of claims 5 to 7 in which a preliminary oxidation step is carried out under conditions such that
30 there is substantially no mid-chain cleavage of the polysaccharide chain.

9. A process according to claim 8 in which the preliminary

oxidation step is carried out in aqueous solution in the presence of periodate at a concentration in the range 1mM to 1M, a pH in the range 3 to 10, a temperature in the range 0 to 60°C and a time in the range 1 min to 48 hours.

5 10. A process according to any preceding claim in which step a) is carried out under conditions such that pendent carboxyl groups on the starting material are not reduced.

 11. A process according to claim 10 in which step a) is carried out in aqueous solution in the presence of borohydride at a concentration in the
10 range 1µM to 0.1M, a pH in the range 6.5 to 10, a temperature in the range 0 to 60°C and a period in the range 1 min to 48 h.

 12. A process according to any preceding claim in which the aldehyde derivative is reacted with a substrate having a primary amine group or a hydrazide group.

15 13. A process according to claim 12 in which the product is reduced.

 14. A process according to claim 12 or claim 13 in which the substrate is a peptide or a protein.

 15. A process according to claim 14 in which the substrate is a
20 peptide therapeutic.

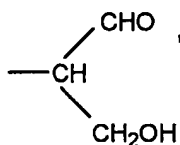
 16. A process according to claim 12 or claim 13 in which the substrate is a compound having a functional group substituent and a dibasic organic group joining the amine or hydrazide group and the functional group.

 17. A process according to claim 16 in which the product is
25 subsequently reacted with a compound having a thiol group, preferably a protein.

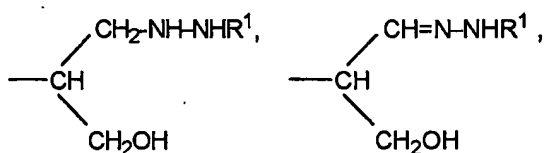
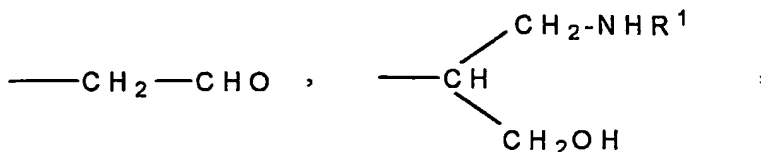
 18. A process according to claim 12 or 13 in which the substrate is a drug delivery system, a cell, preferably a microbial cell or an animal cell, a virus or a synthetic polymer.

30 19. A compound which is an aldehyde derivative of a di-, oligo or poly-saccharide comprising at least one sialic acid unit, in which the terminal

unit at the reducing end includes an aldehyde moiety or is a group OR, in which R is selected from



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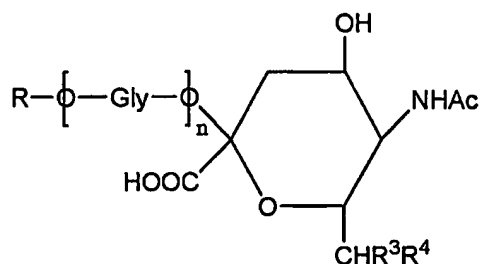
-CH₂CH₂NHR¹, CH₂CH=N-NHR¹ and CH₂CH₂NHNHR¹ in which R¹ is H, C₁₋₂₄ alkyl, aryl C₂₋₆ alkanoyl, or a polypeptide or a protein linked through the N terminal or the γ-amine group of a lysine residue, a drug delivery system or is an organic group having a functional substituent adapted for reaction with a sulfhydryl group.

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20. A compound according to claim 19 in which the compound has a passivated unit at the non-reducing end.

21. A compound according to claim 19 or 20 which has general formula I

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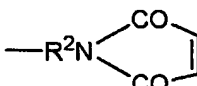


I

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in which R³ and R⁴ are selected from

- i) R^3 is H and R^4 is OH
- ii) where R is $\text{CH}(\text{CHO})\text{CH}_2\text{OH}$ or $-\text{CH}_2\text{CHO}$, R^3 and R^4 together are $=\text{O}$;
- iii) where R is $\text{CH}(\text{CH}_2\text{OH})\text{CH}_2\text{NHR}^1$ or $-\text{CH}_2\text{CH}_2\text{NHR}^1$, R^3 is H and R^4 is $-\text{NHR}^1$;
- iv) where R is $-\text{CH}(\text{CH}_2\text{OH})\text{CH}_2\text{NHNHR}^1$ or $-\text{CH}_2\text{CH}_2\text{NHNHR}^1$, R^3 is H and R^4 is $-\text{NHNHR}^1$; or
- v) $-\text{CH}_2\text{CH}=\text{N}-\text{NHR}^1$, R^3 and R^4 are together $=\text{N}-\text{NHR}^1$;
- Ac is acetyl.
22. A compound according to claim 21 in which R^3 is H and R^4 is OH.
23. A compound according to any of claims 19 to 22 which is a polysaccharide in which substantially all the saccharide units are of sialic acid, joined 2-8, 2-9 or alternating 2-8/2-9, to one another.
24. A compound according to any of claim 23 having at least 2, preferably at least 5, more preferably at least 10, most preferably at least 50, sialic acid units in the polysaccharide chain.
25. A compound according to any of claims 19 to 24 in which R^1 is a protein or peptide or a drug delivery system.
26. A compound according to any of claims 19 to 25 in which R is
- $$\begin{array}{c} \text{CHO} \\ \diagup \\ \text{---CH} \\ \diagdown \\ \text{CH}_2\text{OH} \end{array}$$
27. A compound according to any of claims 19 to 25 in which R is
- $$\begin{array}{c} \text{CH}_2\text{NHR}^1 \\ \diagup \\ \text{---CH} \\ \diagdown \\ \text{CH}_2\text{OH} \end{array}$$
28. A compound according to claim 25 or claim 27 in which R^1 is a peptide or protein therapeutic, preferably an antibody or fragment.

29. A compound according to any of claims 19 to 24 in which R¹ is a group  in which R² is a dibasic organic group, preferably a C₂-

12-alkanediyl group.

5 30. A composition comprising a compound according to any of claims 19 to 29 and a diluent.

31. A pharmaceutical composition comprising a compound according to claim 25 or claim 28 and a pharmaceutically acceptable excipient.

10 32. A process in which a sialic acid starting material having a terminal sialic acid at a non-reducing terminal end is subjected to the following steps:

c) a selective oxidation step to oxidise the non-reducing terminal sialic acid unit at the 7, 8 vicinal diol group to form a 7-aldehyde; and

15 d) a reduction step to reduce the 7-aldehyde group to the corresponding alcohol.

33. A process according to claim 32 in which the starting material is a di-, oligo- or poly-saccharide.

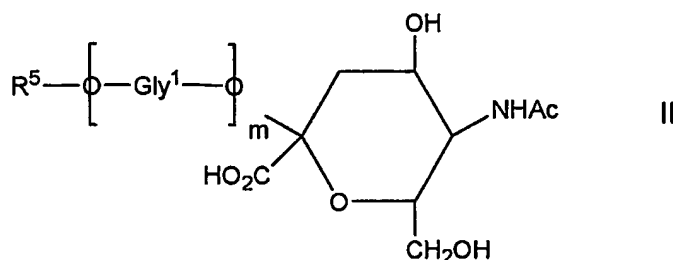
20 34. A process according to claim 33 in which the polysaccharide comprises mid-chain sialic acid units, and preferably consists substantially only of sialic acid units.

25 35. A process according to any of claims 32 to 34 in which the reduction step is followed by an oxidation step which oxidises the reducing terminal unit but does not oxidise the moiety produced in the oxidation and reduction reactions of the non-reducing terminal unit.

30 36. A process according to any of claims 32 to 36 in which oxidation step (c) is carried out in aqueous solution in the presence of periodate at a concentration in the range 1mM to 1M, a pH in the range 3 to 10, a temperature in the range 0 to 60°C and a time in the range 1 min to 48 hours.

37. A process according to any of claims 32 to 37 in which the reduction step d) is carried out in aqueous solution in the presence of borohydride at a concentration in the range $1\mu\text{M}$ to 0.1M , a pH in the range 6.5 to 10, a temperature in the range 0 to 60°C and a period in the range 1 min to 48 h.

38. A compound which has the general formula II



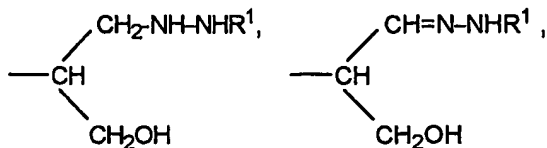
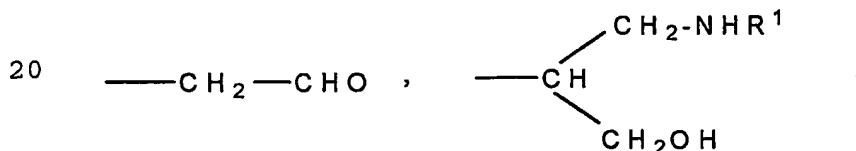
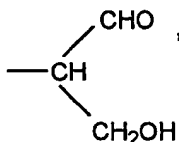
in which Ac is acetyl;

m is 0 or more;

Gly¹O is glycosyl; and

R⁵ is an organic group.

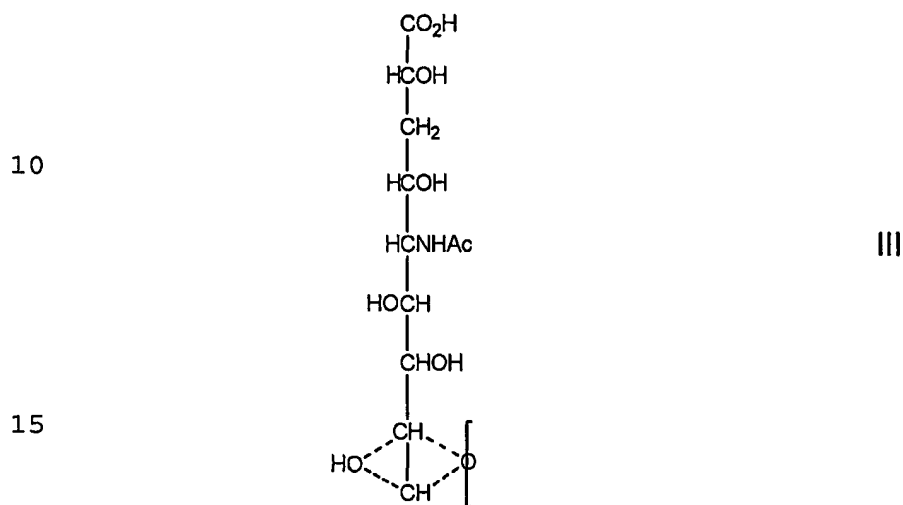
39. A compound according to claim 38 in which R⁵ is in which R is selected from



25 -CH₂CH₂NHR¹, CH₂CH=N-NHR¹ and CH₂CH₂NHNHR¹ in which R¹ is H, C₁₋₂₄

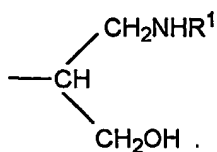
alkyl, aryl C₂₋₆ alkanoyl, or a polypeptide or a protein linked through the N terminal or the γ-amine group of a lysine residue, a drug delivery system or is an organic group having a functional substituent adapted for reaction with a sulfhydryl group.

- 5 40. A compound according to claim 39 in which R⁵ is a group of general formula III,



- 20 41. A compound according to any of claims 38 to 40 in which the groups GlyO comprise, preferably consist substantially only of, sialic acid units.

42. A compound according to claim 39 in which R⁵ is



43. A compound according to claim 42 in which R¹ is a peptide or protein therapeutic, preferably an antibody or fragment.
- 25 44. A pharmaceutical composition comprising a compound according to claim 43 and a pharmaceutical excipient.
45. A composition comprising a compound according to any of claims 38 to 43 and a diluent.